

## Claims:

1. A method for reducing or eliminating the susceptibility of a tropoelastin or a tropoelastin variant to proteolysis comprising mutating a sub-sequence in the tropoelastin or the tropoelastin variant so that the susceptibility of the tropoelastin or the tropoelastin variant to proteolysis is reduced or eliminated.
2. A method according to claim 1 wherein one sub-sequence is mutated.
- 10 3. A method according to claim 1 wherein one amino acid residue in the sub-sequence is mutated.
4. A method according to claim 1 wherein the sub-sequence is capable of being digested by a serine protease.
5. A method according to claim 4 wherein the sub-sequence has an amino acid sequence including the sequence: RAAAG.
- 15 6. A method according to claim 5 wherein the sub-sequence is mutated by replacing arginine in the sequence: RAAAG with alanine.
- 20 7. A method according to claim 4 wherein the sub-sequence has an amino acid sequence selected from the group of sequences shown in SEQ ID NOS: 17 to 44.
8. A method according to claim 7 wherein the sub-sequence is mutated by replacing arginine in the sequence selected from the group of sequences shown in SEQ ID NOS: 17 to 44 with alanine.
- 25 9. A method according to claim 4 wherein the sub-sequence is capable of being digested by thrombin and has an amino acid sequence shown in SEQ ID NOS: 8 or 9.
- 30 10. A method according to claim 4 wherein the sub-sequence is capable of being digested by plasmin and has an amino acid sequence shown in SEQ ID NOS: 11 or 12.
11. A method according to claim 4 wherein the sub-sequence is capable of being digested by kallikrein.
- 35 12. A method according to claim 11 wherein the sub-sequence has an amino acid sequence shown in SEQ ID NOS: 9

or 10.

13. A method according to claim 1 wherein the sub-sequence is capable of being digested by a metalloproteinase.

5 14. A method according to claim 13 wherein the sub-sequence has an amino acid sequence including the sequence: ALAAA.

10 15. A method according to claim 14 wherein the sub-sequence is mutated by replacing alanine at any position in the sequence: ALAAA with another amino acid residue.

16. A method according to claim 15 wherein the sub-sequence is mutated by replacing the alanine which is N-terminal to leucine in the sequence: ALAAA with another amino acid.

15 17. A method according to claim 13 wherein the sub-sequence has an amino acid sequence selected from the group of sequences shown in SEQ ID NOS: 45 to 70.

20 18. A method according to claim 17 wherein the sub-sequence is mutated by replacing alanine at any position in the sequence selected from the group of sequences shown in SEQ ID NOS: 45 to 70 with another amino acid residue.

19. A method according to claim 18 wherein the alanine that is replaced is N-terminal to leucine.

25 20. A method according to claim 13 wherein the sub-sequence is capable of being digested by gelatinase A or B.

21. A method according to claim 20 wherein the sub-sequence has an amino acid sequence shown in SEQ ID NO: 13.

22. A method according to any one of claims 1 to 21 wherein the tropoelastin is human tropoelastin.

30 23. A derivative comprising an amino acid sequence of a tropoelastin or a tropoelastin variant which contains a sub-sequence that is mutated so that the susceptibility of the derivative to proteolysis is reduced or eliminated when compared with the susceptibility of the tropoelastin or the  
35 tropoelastin variant to proteolysis.

24. A derivative according to claim 23 wherein one

sub-sequence is mutated.

25. A derivative according to claim 23 wherein one amino acid residue in the sub-sequence is mutated.

26. A derivative according to claim 23 wherein the  
5 mutated sub-sequence has reduced or eliminated susceptibility to digestion by a serine protease.

27. A derivative according to claim 26 wherein the mutated sub-sequence includes a sequence: RAAAG provided that arginine in the sequence is replaced with alanine.

10 28. A derivative according to claim 26 wherein the mutated sub-sequence has a sequence selected from the group of sequences shown in SEQ ID NOS:17 to 44 provided that arginine in the sequence is replaced with alanine.

29. A derivative according to claim 26 wherein the  
15 mutated sub-sequence has reduced or eliminated susceptibility to digestion by thrombin and has a sequence shown in SEQ ID NOS: 8 or 9 provided that an amino acid residue in the sequence is mutated.

30. A derivative according to claim 26 wherein the  
20 mutated sub-sequence has reduced or eliminated susceptibility to digestion by plasmin and has a sequence shown in SEQ ID NOS: 11 or 12 provided that an amino acid residue in the sequence is mutated.

31. A derivative according to claim 26 wherein the  
25 mutated sub-sequence has reduced or eliminated susceptibility to digestion by kallikrein.

32. A derivative according to claim 31 wherein the mutated sub-sequence has a sequence shown in SEQ ID NOS: 9 or 10 provided that an amino acid residue in the sequence  
30 is mutated.

33. A derivative according to claim 23 wherein the mutated sub-sequence has reduced or eliminated susceptibility to digestion by a metalloproteinase.

34. A derivative according to claim 33 wherein the  
35 mutated sub-sequence includes a sequence: ALAAA provided that alanine at any position in the sequence is replaced

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with any amino acid residue.

35. A derivative according to claim 34 wherein the alanine N-terminal to the leucine is mutated by replacing that alanine with any amino acid residue.

5       36. A derivative according to claim 33 wherein the mutated sub-sequence is a sequence selected from the group of sequences shown in SEQ ID NOS:45 to 70 provided that alanine at any position in the sequence is replaced with any amino acid residue.

10       37. A derivative according to claim 36 wherein the alanine N-terminal to the leucine is mutated by replacing that alanine with any amino acid residue.

38. A derivative according to claim 33 wherein the mutated sub-sequence has reduced or eliminated  
15 susceptibility to digestion by gelatinase A or B.

39. A derivative according to claim 38 wherein the mutated sub-sequence has the sequence shown in SEQ ID NO: 13 provided that at least one amino acid residue in the sequence is mutated.

20       40. A derivative according to claim 39 wherein the sub-sequence has a sequence shown in SEQ ID NO: 13 provided that the alanine N-terminal to the leucine is mutated by replacing that alanine with any amino acid residue.

41. A derivative according to any one of claims 23 to  
25 40 wherein the tropoelastin is human tropoelastin.

42. A method for enhancing the susceptibility of a tropoelastin or a tropoelastin variant to proteolysis comprising inserting a sub-sequence into the tropoelastin or the tropoelastin variant so that the susceptibility of  
30 the tropoelastin or the tropoelastin variant to proteolysis is enhanced.

43. A method according to claim 42 wherein one sub-sequence is inserted.

44. A method according to claim 42 wherein the  
35 inserted sub-sequence is capable of being digested with a serine protease.

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45. A method according to claim 44 wherein the inserted sub-sequence has an amino acid sequence including the sequence: RAAAG.

46. A method according to claim 44 wherein the  
5 inserted sub-sequence has an amino acid sequence selected from the group of sequences shown in SEQ ID NOS: 17 to 44.

47. A method according to claim 44 wherein the inserted sub-sequence is capable of being digested by thrombin and has an amino acid sequence shown in SEQ ID  
10 NOS: 8 or 9.

48. A method according to claim 44 wherein the inserted sub-sequence is capable of being digested by plasmin and has an amino acid sequence shown in SEQ ID NOS:  
11 or 12.

49. A method according to claim 44 wherein the  
15 inserted sub-sequence is capable of being digested by kallikrein.

50. A method according to claim 49 wherein the inserted sub-sequence has an amino acid sequence shown in  
20 SEQ ID NOS: 9 or 10.

51. A method according to claim 42 wherein the inserted sub-sequence is capable of being digested by a metalloproteinase.

52. A method according to claim 51 wherein the  
25 inserted sub-sequence has an amino acid sequence including the sequence: ALAAA.

53. A method according to claim 51 wherein the inserted sub-sequence has an amino acid sequence selected from the group of sequences shown in SEQ ID NOS: 45 to 70.

54. A method according to claim 51 wherein the  
30 inserted sub-sequence is capable of being digested by gelatinase A or B.

55. A method according to claim 54 wherein the inserted sub-sequence has the amino acid sequence shown in  
35 SEQ ID NO: 13.

56. A method according to any one of claims 42 to 55

wherein the tropoelastin is human tropoelastin.

57. A derivative comprising an amino acid sequence of a tropoelastin or a tropoelastin variant which contains an inserted sub-sequence that enhances the susceptibility of the derivative to proteolysis when compared with the susceptibility of the tropoelastin or the tropoelastin variant to proteolysis.

58. A derivative according to claim 57 wherein the amino acid sequence contains one inserted sub-sequence.

59. A derivative according to claim 57 wherein the inserted sub-sequence is capable of being digested by a serine protease.

60. A derivative according to claim 59 wherein the inserted sub-sequence includes the sequence: RAAAG.

61. A derivative according to claim 59 wherein the inserted sub-sequence is a sequence selected from the group of sequences shown in SEQ ID NOS: 17 to 44.

62. A derivative according to claim 59 wherein the inserted sub-sequence is capable of being digested by thrombin and has the sequence shown in SEQ ID NOS: 8 or 9.

63. A derivative according to claim 59 wherein the inserted sub-sequence is capable of being digested by plasmin and has the sequence shown in SEQ ID NOS: 11 or 12.

64. A derivative according to claim 59 wherein the inserted sub-sequence is capable of being digested by kallikrein.

65. A derivative according to claim 64 wherein the inserted sub-sequence has the sequence shown in SEQ ID NOS: 9 or 10.

66. A derivative according to claim 57 wherein the inserted sub-sequence is capable of being digested by a metalloproteinase.

67. A derivative according to claim 66 wherein the inserted sub-sequence includes the sequence: ALAAA.

68. A derivative according to claim 66 wherein the inserted sub-sequence is a sequence selected from the group

of sequences shown in SEQ ID NOS: 45 to 70.

69. A derivative according to claim 66 wherein the inserted sub-sequence is capable of being digested with gelatinase A or B.

5        70. A derivative according to claim 66 wherein the inserted sub-sequence has the sequence shown in SEQ ID NO:13.

71. A derivative according to any one of claims 57 to 70 wherein the tropoelastin is human tropoelastin.

10       72. A method for enhancing the susceptibility of a polypeptide to proteolysis comprising inserting an amino acid sequence which corresponds to a susceptible region of tropoelastin into the polypeptide so that the susceptibility of the polypeptide to proteolysis is  
15 enhanced.

73. A polypeptide derivative exhibiting enhanced susceptibility to proteolysis in comparison with a corresponding polypeptide, the derivative characterised in that an amino acid sequence which corresponds to an amino  
20 acid sequence in a susceptible region of tropoelastin is inserted into the polypeptide so that the susceptibility of the polypeptide to proteolysis is enhanced.

74. A method for producing a chimeric derivative which comprises linking a derivative according to any one of  
25 claims 23, 57 or 73 with a peptide via an amino acid sequence which corresponds to an amino acid sequence in a susceptible region of tropoelastin.

75. A chimeric derivative comprising a derivative according to any one of claims 23, 57 and 73 which is  
30 linked with a peptide domain via an amino acid sequence which corresponds to an amino acid sequence in a susceptible region of tropoelastin.

76. A peptidomimetic molecule comprising all or part of a peptide selected from the group consisting of  
35 KAPGVGGAF, RAAAGLG, RSLSPELREGD, KAAQFGLVPGV, KSAAKVAAKAQLRAA, RSLSPELRE and LAAAKAAKYGAA.

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77. A peptidomimetic molecule which has the sequence:  
H-Ala-Ala-Lys-Ala-Gln-Leu-Arg-Ala-Ala-Ala-Gly-Leu-Gly-Ala-  
OH or H-Ala-Ala-Lys-Ala-Gln-Leu-Arg-R-Ala-Ala-Ala-Gly-Leu-  
Gly-Ala-OH (where R = a reduced peptide bond).

5 78. A peptidomimetic molecule which is a retro-inverso  
pseudo peptide which has the sequence: H-D-Ala-Gly-D-Leu-  
Gly-D-Ala-D-Ala-D-Ala-(R)-D-Arg-D-Leu-D-Gln-D-Ala-D-Lys-D-  
Ala-D-Ala-OH (where R = a reduced peptide bond) or H-D-Ala-  
Gly-D-Leu-Gly-D-Ala-D-Ala-D-Ala-D-Arg-D-Leu-D-Gln-D-Ala-D-  
10 Lys-D-Ala-D-Ala-OH.

79. A peptidomimetic molecule which has the sequence  
H-Val-Pro-Gly-Ala-Leu-Ala-Ala-Ala-OH or H-Val-Pro-Gly-Ala-  
(R)-Leu-Ala-Ala-Ala-OH (where R = a reduced peptide bond)

80. A peptidomimetic molecule which is a retro-inverso  
15 pseudo peptide which has the sequence:: H-D-Ala-D-Ala-D-  
Ala-D-Leu-(R)-D-Ala-Gly-D-Pro-D-Val-OH (where R = a reduced  
peptide bond) or H-D-Ala-D-Ala-D-Ala-D-Leu-D-Ala-Gly-D-Pro-  
D-Val-OH.

81. A method for enhancing the purification of a  
20 tropoelastin or a tropoelastin variant comprising including  
a peptidomimetic molecule according to any one of claims 76  
to 80 in a crude tropoelastin or tropoelastin variant  
preparation which is being subjected to purification.

82. A pharmaceutical composition comprising a  
25 derivative according to any one of claims 23, 57, 73 and  
75, or a peptidomimetic molecule according to any one of  
claims 76 to 80 and a pharmaceutically acceptable carrier.

83. A nucleotide sequence encoding a derivative  
according to any one of claims 23, 57, 73 and 75.

30 84. A cell containing a nucleotide sequence according  
to claim 83.

85. A method of producing a derivative according to  
any one of claims 23, 57, 73 and 75 comprising maintaining  
a cell according to claim 85 in conditions suitable for the  
35 production of the derivative.

86. An implant comprising a derivative according to



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any one of claims 23, 57, 73 and 75.